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Stability and phase behavior of fish oil emulsion containing konjac glucomannan in goat milk systems

Siriwan Suknicom[1] and Chaleeda Borompichaichartkul[1,2*]

[1]Department of Food Technology, Faculty of Science, Chulalongkorn University, 10330, Bangkok, Thailand
[2]Emerging Process for Food Functionality design (EPFFD) research unit, Department of Food Technology, Faculty of Science, Chulalongkorn University, 10330, Bangkok, Thailand

*Corresponding author; E-mail: chaleeda.b@chula.ac.th

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Abstract

We investigated the effects of konjac glucomannan (KGM) solution (0.02%-0.5%, w/w) at different pH (7-10) on the stability of KGM-milk emulsion containing of 5% fish oil. Particle size in the emulsion was increased with the increase in pH values of KGM solution above 7 at all concentrations of KGM. Zeta potential of the emulsion was increased when the pH of KGM solution was increased at all concentration of KGM. The precipitation percentage (0.02%-0.5%) of KGM at any pH was 0%, which stabilized the mixture up to 2 days. However, when the concentration of KGM in the emulsion exceeded 0.5% w/w, precipitation occurred rapidly. The increase in pH values from 7-10 at the same concentration of KGM could increase stability of the emulsion. Confocal laser scanning microscopy images confirmed the assumption that microstructures of KGM-stabilized emulsions were controlled by pH. The images revealed that lowering the pH resulted in expanded appearance of the aggregates. Moreover, the appearance of aggregates changed from isolated cluster to cluster networks, as shown in the emulsion at pH 7 compared to the emulsions at pH 10. Fish oil emulsion containing KGM in milk at different pH and concentration of KGM solution exhibited differences in stability. The mixture stability was enhanced when KGM concentration in solution was decreased and when the pH was increased. The highest stability of the mixture was obtained with 0.02% and 0.04% KGM at pH 9 and 10. The stability of the emulsion with different conditions was due to the merging of steric and electrostatic stabilization.

Keywords**:** *emulsion; fish oil; goat milk; konjac glucomannan; pH; phase behavior; stability.*

1. Introduction

Emulsion is a suspension formed by a spherical liquid droplet, dispersing in other liquids that cannot be combined (Hoffmann & Reger, 2014). It is widely used in foods to encapsulate various substances, such as biologically active substances (McClements, 2012). An oil-in-water emulsion consists of three parts: the oil phase, aqueous phase, and surface-active substances. The oil phase is characterized by a droplet within the water phase. The aqueous phase and surface-active substances are two phases that cannot be changed (Dalgleish, 2006). Emulsions are always thermodynamically unstable but can become stable through physical mechanisms, including partial agglomeration, precipitation, Oswald ripening, agglomeration, phase inversion, creaming, and flocculation (McClements & Jafari, 2018). Many parameters influence the lifetime of emulsion, including emulsion droplet size, preparation process, such as homogenizer type, amount, and type of components used (Romero, Felix, Perez-Puyana, Choplin, & Guerrero, 2017). There are several common natural emulsifiers, such as

protein, polysaccharides, and phospholipid, which are used reduce the surface tension in an emulsion system, thereby resulting in a stable emulsion (Ozturk & McClements, 2016). The polysaccharides in emulsions have an influence on the rheological properties and the protein-stable interface due to its extensive availability, good chemical and physical stability, and low cost (Wang, Liu & Qin, 2017). Previous studies have suggested that the use of polysaccharides as stabilizers of the aqueous phase can increase the emulsion stability, with polysaccharides increasing the stabilization of the proteins in the emulsion by creating a polysaccharide–protein double-layer interface (Dickinson, 2011; Mao, Roos & Miao, 2015). In addition, polysaccharides are able to form a network structure in the aqueous phase, which can limit the movement of oil droplets by steric hindrance and enhance the emulsion creaming stability (Lin et al., 2017).

Konjac Glucomannan (KGM) is a type of water-soluble polysaccharide with a molecular weight ranging from 200-2,000 kDa (Zhang, Chen & Yang, 2014). In general, KGM can be extracted from konjac tubes, which is the genus Amorphophallus (Fang & Wu, 2004). The molecular structure of KGM contains D-mannose and D-glucose in a molar ratio of 1.6 to 1, respectively. The molecules are linked by β-(1,4) glycosidic linkages. It also has side branches and acetyl groups are randomly present in the C-6 position of the sugar (Yoshimura & Nishinari, 1999). KGM can form a highly viscous solution when dissolved in water, making it a gelling agent, which is approved as a food additive by the U.S. Food and Drug Administration (Chua, Baldwin, Hocking, & Chan, 2010). In addition, KGM has several potent nutritional properties, including its ability to lower blood cholesterol and blood sugar levels. It is helpful for weight loss and promotes intestinal activity and immune response (Zhang, Xie, & Gan, 2005). These characters make KGM a crucial food additive for use in milk protein-stabilized emulsion (Dai, Jiang, Shah, & Corke, 2017). Studies have shown that the acetyl groups on KGM structure are associated with its water soluble and gelling properties. Exposure to alkaline compounds can cause deacetylation of KGM, thereby significantly reducing its solubility in water. Degree of deacetylation (DD) also affects the physico-chemical and gelation properties of KGM. Water solubility decreases with increases in DD and the hydrophobic interactions present in KGM gel are strengthened (Du, Li, Che, & Li, 2012). However, few studies have been conducted on the use of KGM in the modification of the aqueous phase and emulsion stability.

2. Objectives

This study evaluated the related parameters of KGM at different pH and concentrations to understand the behavior and interactions of KGM polymer chains in stabilizing emulsion of oil in milk system.

3. Materials and methods

3.1 Materials

Fresh goat's milk containing 3.5% protein, 3.0% total fat, and 4.3% lactose were used. KGM powder was sourced from Yunnan Genyun Konjac Resource Corp., Kunming (Yunnan, PR China). Fish oils (food grade, DHA+EPA approximately 70%) were purchased from TC Union Agrotech Co., Ltd., Thailand.

3.2 Sample preparation

KGM powder was dissolved in water at a concentration of 0.1%-0.5% (w/w) by continuous stirring for 4 hours at room temperature. The pH of the KGM solution was then adjusted to 7.0, 8.0, 9.0, and 10.0 using 0.1 M NaOH and 0.1 M HCl. The amount of NaOH or HCl added to the KGM solution for the pH adjustment was very low and it therefore had little effect on the concentration of the KGM solution. The pH-adjusted solution was kept at room temperature for 12 hours to allow the KGM to swell and make the pH stable. Afterward, milk, fish oil, and the prepared KGM solution were mixed using overhead homogenizer (Ultra-Turrax T-25, IKA, Malaysia) for 1 minute. The final composition of the mixture was 5% fish oil, varying KGM content (0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.3, 0.4, and 0.5%) at pH of 7, 8, 9, and 10, and 3% milk protein. The coarse emulsions were immediately homogenized by passing in a single stage high pressure homogenizer (APV 2000, APV, Denmark) at 40 MPa (400 bar) for two times. The freshly prepared emulsion was added with sodium azide (0.02% v / v), an antimicrobial agent.

3.3 Particle size analysis

The particle size of all emulsions was determined by dynamic light scattering using a

Zetasizer (Zetasizer Nano ZSP, Malvern, United Kingdom). The emulsions were dispersed with distilled water before measurement and the particle size was measured in styrene cuvette. The Z-average of all emulsion exhibited the size of the particle. Z-average was analyzed immediately after preparation of the emulsion and every 2 days during storage at 4°C.

3.4 Zeta potential measurements

The zeta potential values of all emulsions were measured with a Zetasizer instrument (Zetasizer Nano ZSP, Malvern, United Kingdom). Zeta potential values were measured immediately after preparation of the emulsion and every 2 days during storage at 4^0C.

3.5 Precipitation percentage

Precipitation percentages of all emulsions were examined according to Ye & Harte (2014) with some modifications. Visual observation was performed after the emulsion had been stored at 4°C. When the phase separation was observed, the precipitation of the suspension was calculated by the following formula:

$$\% \text{ Separation } = (Vp/Vt) \times 100$$

Where: *Vp* represents the volume (ml) of the layer at which the precipitation occurs and *Vt* represents the total volume (ml) of the emulsion.

3.6 Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) was examined according to Li & Shah (2015) with some modifications. The polysaccharide/protein staining was achieved by mixing 1 mL emulsion with 10 mL of Nile Red (1 mg/mL in ethanol) for 5 minutes. Afterward, 10 mL of fluorescein isothiocyanate (FITC; 1 mg/mL in ethanol) was added and the mixture was stirred for 5 min. To prepare CLSM samples, 1 mL of the stained emulsion was transferred onto microscope slide and cover slips were used to cover the emulsions. These samples were examined using a CLSM (FLUOVIEW FV10i, Olympus, Japan). FITC and Nile Red were excited by 488 nm laser and 534 nm laser, respectively. The emitted lights of FITC and Nile Red were set at 493-538 nm and 586-753 nm, respectively.

3.7 Data analysis

All experiments were performed in triplicate. Results were presented as mean values ± standard deviation (SD). Data were analyzed by one-way analysis of variance with Duncan's multiple range tests using IBM SPSS Statistics for Windows, Version 22 (SPSS Inc, Chicago, USA) and significant differences were accepted at $p<0.05$.

4. Results and discussion

4.1 Particle size analysis

Table 1 shows the effects of KGM concentration and pH values on particle size. On day 0, the particle size of the emulsion with a KGM concentration from 0.02% (w/w) to 0.5% (w/w) at pH 7, 8, 9, and 10 was approximately 900 nm. After day 2, the increase of particle size was noticeable when KGM concentration was higher than 0.3% (w/w). For instance, the increase of particle size after 2 days at pH 7 was 40.87% when KGM concentration was 0.3% (w/w), whereas the increase of particle size was 3.32% when KGM concentration was 0.02% (w/w). An increase in pH from 7 to 10 results in slower rate of particle size increase across all KGM concentrations. For example, the increase of particle size after 4 days of storage was 27.72% at pH 7, whereas at pH 10, it dropped to 22.48% with 0.04%KGM concentration. This result indicates that the particle size of the emulsion depends on the KGM concentration and pH values. Similar results were reported by Dai et al. (2017) for KGM-milk mixtures, demonstrating that the stability of the mixture increases with increase in the milk percentage and decrease in concentration of KGM solution. KGM is a natural polysaccharide that is dispersible in water and forms a network structure with different particle sizes, depending on the concentration. As KGM concentration increases, more network structures are created, resulting in larger particle sizes. There is another possibility to explain the change in droplet size of KGM emulsion, which is related to the effect of pH. Reducing the droplet size under alkaline conditions may reflect the effect of increased NaOH content in the oil phase itself, which facilitates the emulsification process and releases fatty acids if triglycerides are broken down with high NaOH levels, thus resulting in small droplets at high pH (Marinova et al., 1996). Similar results were reported by Hosseini-Parvar, Osano and Matia-Merino (2016) for basil seed gum used to stabilize 30% (w/w) oil-in-water emulsions. The researchers reported that emulsions stabilized by polysaccharides will undergo coalescence or flocculation at the pH where the carbohydrate part has lost all its net charge.

Table 1 Particle sizes (nm) obtained from the use of different pH (7, 8, 9, and 10) and KGM concentrations

KGM concentration (%)	pH	Day					
		0	2	4	6	8	10
0.02	7	960 ± 14^{Bcde}	993 ± 11^{Bfg}	1012 ± 1^{Bl}	1253 ± 137^{Aef}	1309 ± 16^{Abc}	-
	8	909 ± 11^{Edef}	1007 ± 9^{Dfg}	1172 ± 51^{Cjk}	1273 ± 40^{Bef}	1331 ± 6^{Ab}	-
	9	908 ± 17^{Ddef}	968 ± 54^{Cfg}	1161 ± 46^{Bjk}	1296 ± 8^{Acde}	1297 ± 5^{Ac}	1332 ± 8^{Ac}
	10	904 ± 17^{Def}	956 ± 48^{DCg}	1017 ± 4^{Cl}	1135 ± 63^{Bh}	1196 ± 7^{Be}	1307 ± 228^{Ad}
0.04	7	962 ± 15^{Cbc}	999 ± 1^{BCfg}	1331 ± 52^{Bl}	1306 ± 48^{Acde}	1331 ± 52^{Ab}	-
	8	920 ± 17^{Dcde}	101 ± 1^{Cfg}	1270 ± 33^{Bhij}	1305 ± 15^{Bcde}	1417 ± 27^{Aa}	-
	9	938 ± 30^{Ecde}	1006 ± 11^{Dfg}	1215 ± 81^{Cijk}	1296 ± 6^{Bcde}	1306 ± 5^{Bbc}	1439 ± 15^{Aa}
	10	931 ± 47^{Ecde}	1011 ± 7^{Dfg}	1201 ± 3^{Cijk}	1143 ± 48^{Bh}	1208 ± 14^{Be}	1419 ± 35^{Ab}
0.06	7	918 ± 23^{Ccde}	1034 ± 57^{CBfg}	1123 ± 101^{Bkl}	1285 ± 30^{Adef}	-	-
	8	937 ± 41^{Ccde}	1014 ± 3^{Bfg}	1302 ± 4^{Aghi}	1342 ± 57^{Acd}	-	-
	9	936 ± 32^{Dcde}	1012 ± 5^{Cfg}	1260 ± 33^{Bhij}	1307 ± 4^{Acde}	1335 ± 6^{Ab}	-
	10	928 ± 22^{Dcde}	1013 ± 4^{Cfg}	1189 ± 8^{Bijk}	1202 ± 6^{Bg}	1226 ± 3^{Ad}	-
0.08	7	950 ± 5^{Bcde}	1001 ± 3^{Bf}	1259 ± 57^{Ahij}	1329 ± 47^{Abcd}	-	-
	8	929 ± 9^{Ccde}	1047 ± 47^{Bfg}	1366 ± 56^{Aefg}	1372 ± 10^{Ab}	-	-
	9	931 ± 29^{Dcde}	1045 ± 57^{Cfg}	1306 ± 1^{Bghi}	1343 ± 38^{Bbc}	1419 ± 25^{Aa}	-
	10	942 ± 34^{Dcde}	1020 ± 1^{Cfg}	1201 ± 3^{Bijk}	1235 ± 53^{Bfg}	1404 ± 60^{Aa}	-
0.1	7	929 ± 3^{Bcde}	1029 ± 3^{Bfg}	1454 ± 179^{Ade}	-	-	-
	8	858 ± 24^{Bf}	1492 ± 11^{Aabc}	1539 ± 129^{Abc}	-	-	-
	9	940 ± 18^{Ccde}	1206 ± 74^{Bd}	1414 ± 101^{Aef}	1425 ± 56^{Aa}	1429 ± 34^{Aa}	-
	10	961 ± 22^{Ccde}	1189 ± 54^{Bd}	1248 ± 41^{Bhij}	1363 ± 47^{Ab}	1431 ± 25^{Aa}	-
0.2	7	969 ± 25^{Bab}	1169 ± 147^{Bde}	1543 ± 188^{Abc}	-	-	-
	8	904 ± 43^{Bef}	1571 ± 18^{Aab}	1636 ± 84^{Aa}	-	-	-
	9	911 ± 86^{Bcde}	1211 ± 13^{Ad}	1305 ± 5^{Aghi}	-	-	-
	10	964 ± 13^{Bbc}	1240 ± 37^{Ad}	1326 ± 70^{Afgh}	-	-	-
0.3	7	965 ± 21^{Bbc}	1632 ± 40^{Aa}	-	-	-	-
	8	958 ± 41^{Bcde}	1525 ± 147^{Aabc}	-	-	-	-
	9	954 ± 6^{Bcde}	1487 ± 144^{Abc}	-	-	-	-
	10	952 ± 40^{Bcde}	1419 ± 87^{Aef}	-	-	-	-
0.4	7	967 ± 15^{Bbc}	1433 ± 59^{Abc}	-	-	-	-
	8	940 ± 32^{Bcde}	1540 ± 125^{Aab}	-	-	-	-
	9	991 ± 7^{Bab}	1525 ± 60^{Aabc}	-	-	-	-
	10	954 ± 25^{Bcde}	1388 ± 62^{Ac}	-	-	-	-
0.5	7	970 ± 12^{Bab}	1570 ± 12^{Aab}	-	-	-	-
	8	965 ± 23^{Bbc}	1532 ± 43^{Aabc}	-	-	-	-
	9	956 ± 4^{Bcde}	1536 ± 123^{Aabc}	-	-	-	-
	10	954 ± 14^{Bcde}	1420 ± 42^{Abc}	-	-	-	-

All values are presented as the mean ± SD.

Mean values followed by different superscripted letters in the same row (A–D) or column (a–l) are significantly different, according to Duncan's multiple range test ($p < 0.05$).

- is not analyzed due to the products deterioration

Table 2 Zeta potential (mV) obtained from the use of different pH (7, 8, 9, and 10) and KGM concentrations

KGM concentration (%)	pH	Day					
		0	2	4	6	8	10
0.02	7	$-34.51^{Aghi} \pm 1.10$	$-24.40^{Bhij} \pm 0.70$	$-19.38^{Ck} \pm 0.31$	$-19.24^{Cd} \pm 0.67$	$-15.49^{Df} \pm 0.94$	-
	8	$-36.25^{Acde} \pm 0.33$	$-25.03^{Befg} \pm 1.00$	$-20.55^{Chi} \pm 0.01$	$-20.26^{Ccd} \pm 0.67$	$-16.94^{De} \pm 1.52$	-
	9	$-36.56^{Abcd} \pm 0.48$	$-25.75^{Bab} \pm 0.37$	$-21.63^{Ccde} \pm 0.32$	$-20.93^{CDbc} \pm 0.40$	$-20.11^{Dabc} \pm 0.31$	$-17.14^{Eb} \pm 1.02$
	10	$-37.52^{Aab} \pm 0.48$	$-25.50^{Babc} \pm 0.54$	$-23.85^{Ba} \pm 0.76$	$-21.92^{Cab} \pm 0.78$	$-20.78^{Ca} \pm 0.76$	$-18.13^{Da} \pm 1.54$
0.04	7	$-33.48^{Aj} \pm 0.63$	$-24.44^{Bhij} \pm 0.49$	$-19.74^{Cjk} \pm 0.22$	$-19.55^{Cd} \pm 0.46$	$-16.60^{Def} \pm 2.37$	-
	8	$-35.98^{Adef} \pm 0.50$	$-25.00^{Befg} \pm 0.09$	$-20.95^{Cgh} \pm 0.07$	$-20.37^{Ccd} \pm 0.35$	$-18.75^{Dcd} \pm 0.68$	-
	9	$-36.94^{Aabc} \pm 0.09$	$-25.27^{Bcde} \pm 0.62$	$-21.37^{Cdef} \pm 0.10$	$-20.21^{Ccd} \pm 0.58$	$-20.18^{Cabc} \pm 0.11$	$-18.02^{Da} \pm 1.61$
	10	$-36.97^{Aabc} \pm 0.14$	$-25.23^{Bcde} \pm 0.69$	$-23.70^{Ba} \pm 0.24$	$-21.75^{Cb} \pm 1.07$	$-20.67^{Cab} \pm 1.53$	$-17.61^{Dab} \pm 0.67$
0.06	7	$-34.28^{Aij} \pm 0.54$	$-25.17^{Befg} \pm 0.28$	$-19.27^{Ck} \pm 0.52$	$-19.35^{Cd} \pm 0.14$	-	-
	8	$-35.46^{Aghi} \pm 0.85$	$-25.79^{Bab} \pm 0.31$	$-21.12^{Cgh} \pm 0.24$	$-20.29^{Ccd} \pm 0.35$	-	-
	9	$-36.80^{Abcd} \pm 0.28$	$-25.82^{Ba} \pm 0.23$	$-22.20^{Ccde} \pm 0.43$	$-21.57^{Cb} \pm 0.80$	$-18.31^{Dd} \pm 1.73$	-
	10	$-37.14^{Aabc} \pm 0.98$	$-24.75^{Bfgh} \pm 0.27$	$-23.54^{Bab} \pm 1.56$	$-22.00^{Cab} \pm 1.32$	$-20.06^{Cabc} \pm 0.60$	-
0.08	7	$-34.58^{Aghi} \pm 0.97$	$-24.96^{Befg} \pm 0.08$	$-19.48^{Ck} \pm 0.50$	$-19.64^{Cd} \pm 0.68$	-	-
	8	$-36.28^{Abcd} \pm 0.22$	$-25.34^{Bbcd} \pm 1.07$	$-20.94^{Cgh} \pm 0.50$	$-20.16^{Ccd} \pm 0.26$	-	-
	9	$-36.77^{Abcd} \pm 0.30$	$-25.62^{Babc} \pm 0.36$	$-22.27^{Ccde} \pm 0.22$	$-21.24^{Cbc} \pm 1.28$	$-20.60^{Dbc} \pm 4.58$	-
	10	$-37.36^{Aab} \pm 1.10$	$-25.28^{Bcde} \pm 0.55$	$-23.45^{Cab} \pm 0.81$	$-22.94^{Ca} \pm 1.72$	$-19.60^{Dbc} \pm 1.26$	-
0.1	7	$-33.88^{Aj} \pm 0.23$	$-23.30^{Bk} \pm 0.08$	$-19.98^{Cjk} \pm 0.08$	-	-	-
	8	$-35.74^{Afg} \pm 0.48$	$-24.23^{Bijk} \pm 0.53$	$-21.29^{Cfgh} \pm 0.27$	-	-	-
	9	$-37.63^{Aab} \pm 0.74$	$-24.59^{Bghi} \pm 0.46$	$-22.49^{Ccd} \pm 0.37$	$-21.02^{Cbc} \pm 1.03$	$-19.22^{Dbcd} \pm 1.15$	-
	10	$-37.66^{Aa} \pm 0.34$	$-24.09^{Bijk} \pm 1.18$	$-24.13^{Ba} \pm 1.16$	$-21.66^{Cb} \pm 0.56$	$-19.73^{Cbc} \pm 0.65$	-
0.2	7	$-33.47^{Aj} \pm 0.46$	$-23.67^{Bijk} \pm 0.47$	$-19.28^{Ck} \pm 0.44$	-	-	-
	8	$-35.81^{Aefg} \pm 0.74$	$-24.28^{Bijk} \pm 0.81$	$-20.95^{Cgh} \pm 0.81$	-	-	-
	9	$-36.45^{Abcd} \pm 1.94$	$-25.76^{Bab} \pm 0.36$	$-22.78^{Cbc} \pm 0.94$	-	-	-
	10	$-37.37^{Aab} \pm 0.27$	$-25.09^{Befg} \pm 0.42$	$-24.28^{Ba} \pm 1.03$	-	-	-
0.3	7	$-34.42^{Aghi} \pm 0.19$	$-23.85^{Bijk} \pm 0.49$	-	-	-	-
	8	$-34.32^{Aij} \pm 0.89$	$-24.78^{Bfgh} \pm 0.77$	-	-	-	-
	9	$-36.67^{Abcd} \pm 0.64$	$-24.54^{Bghi} \pm 0.48$	-	-	-	-
	10	$-37.46^{Aab} \pm 0.54$	$-25.05^{Befg} \pm 0.81$	-	-	-	-
0.4	7	$-33.66^{Aj} \pm 0.82$	$-23.87^{Bijk} \pm 0.53$	-	-	-	-
	8	$-34.74^{Aghi} \pm 0.90$	$-24.57^{Bghi} \pm 0.28$	-	-	-	-
	9	$-36.70^{Abcd} \pm 1.07$	$-24.52^{Bghi} \pm 0.31$	-	-	-	-
	10	$-37.17^{Aabc} \pm 0.55$	$-25.46^{Babc} \pm 0.40$	-	-	-	-
0.5	7	$-33.70^{Aj} \pm 0.35$	$-23.51^{Bjk} \pm 0.13$	-	-	-	-
	8	$-35.63^{Agh} \pm 0.33$	$-24.68^{Bfgh} \pm 0.61$	-	-	-	-
	9	$-36.36^{Abcd} \pm 0.61$	$-24.49^{Bhij} \pm 0.34$	-	-	-	-
	10	$-37.40^{Aab} \pm 0.31$	$-25.33^{Bcde} \pm 0.36$	-	-	-	-

All values are presented as the mean ± SD.

Mean values followed by different superscripted letters in the same row (A–D) or column (a–l) are significantly different, according to Duncan's multiple range test ($p < 0.05$).

- is not analyzed due to the products deterioration

4.2 Zeta potential

Emulsion stability depends on the attraction and repulsion between the particles. The particle surface charge determines the attractive and repulsive forces and plays an important role in the stability of the emulsion. Zeta potential is the parameter that evaluates the surface charge to assess the stability of the emulsion. Emulsions with zeta potential higher than +30 mV or less than −30 mV are stable due to the repulsive forces that maintain the particles during dispersion. The emulsion between milk and fish oil without KGM was rather unstable, with zeta potential between -30 mV and +30 mV (−0.88 mV). Dai et al. (2017) reported similar results for KGM-milk mixtures. They demonstrated that milk had a higher absolute zeta potential than KGM-milk mixtures, indicating that milk with KGM was less stable than milk without KGM. However, when the KGM solution was added at different concentrations and pH, the zeta potential of all emulsions in the study at day 0 was negative and less than −30 mV, indicating that the emulsion after mixing was stable. Nevertheless, zeta potential of the emulsion was changed after storage. The increase of zeta potential during storage may be due to reduced electrostatic repulsion and interfacial film strength formed by increasing concentration of KGM, which led to the increase in droplets size and decrease in the emulsion's stability. The effects of KGM concentration and pH values on the zeta potential are shown in Table 2. The increase of KGM concentration at any given pH values slightly affected the zeta potential of the emulsion. Similarly, the increase in pH values from 7 to 10 also slightly affected in the change of zeta potential at all concentration of KGM. Increasing zeta potential leads to a reduction of the stability of mixtures. Similar results were reported in a study by Wang et al., (2020), highlighting that the addition of 0.05-0.15 wt% of soy hull polysaccharide in oil-in-water emulsion increased the zeta potential and significantly reduced the stability of emulsions. The results showed that the amount of biopolymer adsorbed at the oil–water interface increased or the change of the bio-absorbable polymers at the oil and water interface has occurred (Zhao et al., 2015; Huang et al., 2019).

4.3 Precipitation percentage

Emulsions are classified as unstable thermodynamic systems. Aggregation and flocculation of oil droplets are among the factors that cause emulsion instability. Precipitation percentage refers to the volume percentage of the precipitation in emulsion from mixing to separation, which is an observed evidence of the stability of the emulsion (Ye & Harte, 2014). A value of 0% precipitation means emulsion was not separated.

Table 3 shows the effect of KGM concentration and pH values on the precipitation percentage of samples. The precipitation percentage for 0.02–0.5% KGM at any pH was 0%, indicating a stable mixture after mixing for up to 2 days. When the concentration of KGM in the emulsion increased, the separation occurred more rapidly and the precipitation percentage increased. For instance, when the KGM concentration was 0.02% (w/w) at pH 7, the precipitation percentage was 0% until day 8, increasing to 7.39% at day 10, which indicates that the emulsion was stable for 8 days before separation. Meanwhile, with 0.2% KGM at pH 7, the precipitation percentage was 0% until day 4, increasing to 6.85% on day 6, which indicates that the emulsion was stable for 4 days before separation. Consistent with the influence of KGM concentration, in the present study, pH values also affected the precipitation percentage. The increase in pH values from 7-10 at the same concentration of KGM led to an increase of the stability of the emulsion. For instance, at pH 7, 0.04% KGM concentration precipitation percentage was 7.05% on day 10, whereas at pH 10 and the same KGM concentration, the precipitation percentage was 6.96% on day 12.

The KGM molecule is long and it contains acetyl groups, which are hydrophilic. In the milk-KGM emulsion, under certain conditions, the KGM molecules may bond with water molecules in the milk. For this reason, precipitation of protein particles occurs (Dai et al., 2017). However, with increasing pH values of KGM solution, the stability of the emulsion increases. The stability of emulsion is increasing as a function of the solubility of KGM, which depends on the degree of acetylation. Du et al., (2012) found that water solubility of KGM decreased with increased DD (acetyl groups decreased). KGMs with chain-like molecular structures are able to fill the gap between free protein molecules and oil droplets in the emulsion. These KGMs may help to separate the oil droplets, thereby reducing the Brownian-motion-induced contact of proteins and oil droplets (Lu, Zheng, & Miao, 2018).

To summarize the above data, the results shown in Tables 1, 2, and 3 are related. It was found that the stability of the emulsion depends on the particle size and zeta potential. The emulsion has high stability when the particle size and zeta potential are reduced.

Table 3 Effects of KGM concentration and pH on the precipitation percentage of samples

KGM concentration (%)	pH	Day					
		2	4	6	8	10	12
0.02	7	0	0	0	0	7.39 ± 0.10	-
	8	0	0	0	0	7.38 ± 0.03	-
	9	0	0	0	0	0	9.10 ± 0.56
	10	0	0	0	0	0	6.59 ± 0.61
0.04	7	0	0	0	0	7.05 ± 0.57	-
	8	0	0	0	0	8.25 ± 0.14	-
	9	0	0	0	0	0	8.35 ± 1.35
	10	0	0	0	0	0	6.96 ± 0.58
0.06	7	0	0	0	7.61 ± 0.32	-	-
	8	0	0	0	7.06 ± 0.66	-	-
	9	0	0	0	0	7.48 ± 0.34	-
	10	0	0	0	0	8.29 ± 0.10	-
0.08	7	0	0	0	7.62 ± 0.71	-	-
	8	0	0	0	7.56 ± 0.27	-	-
	9	0	0	0	0	8.29 ± 0.01	-
	10	0	0	0	0	8.39 ± 0.10	-
0.1	7	0	0	6.70 ± 0.52	-	-	-
	8	0	0	7.36 ± 0.10	-	-	-
	9	0	0	0	0	8.61 ± 1.22	-
	10	0	0	0	0	7.19 ± 0.01	-
0.2	7	0	0	6.85 ± 0.55	-	-	-
	8	0	0	6.81 ± 0.59	-	-	-
	9	0	0	7.09 ± 0.69	-	-	-
	10	0	0	7.04 ± 1.23	-	-	-
0.3	7	0	5.23 ± 0.67	-	-	-	-
	8	0	5.87 ± 0.80	-	-	-	-
	9	0	6.40 ± 0.58	-	-	-	-
	10	0	5.95 ± 0.40	-	-	-	-
0.4	7	0	5.98 ± 0.59	-	-	-	-
	8	0	6.53 ± 0.38	-	-	-	-
	9	0	6.32 ± 0.47	-	-	-	-
	10	0	6.10 ± 0.45	-	-	-	-
0.5	7	0	5.32 ± 0.03	-	-	-	-
	8	0	6.43 ± 0.74	-	-	-	-
	9	0	6.34 ± 0.23	-	-	-	-
	10	0	6.40 ± 0.10	-	-	-	-

All values are presented as the mean ± SD
0 = no separation and - = not analyzed

4.4 Microscopic structure

Nile Red and FITC were used to stain fat globules and protein in milk or dairy products, respectively (Gaygadzhiev, Hill, & Corredig, 2019). However, FITC was used to label polysaccharides (Tromp, van de Velde, van Riel, & Paques, 2001). The orange or red in the picture represents milk protein, while the green picture represents the KGM rich area. The results are consistent with the phenomenon of micellar casein-konjac mixture (1.7% fat and 77.4% protein) that the konjac-FITC conjugate (green channel) shows homogeneity in the solution and mixtures separated in protein (red channel) and konjac (green phase) domains (Dai et al., 2017; Abhyankar, Mulvihill, Chaurin, & Auty, 2011). Emulsion stability can be analyzed by shape, size, and connection of the aggregate structures of phase separation dynamics

theory (Bates, 1999). The nucleation structure and growth are the tiny droplets of the minor phase, which are developed by diffusion of the component from the supersaturated continuum. This happens in the metastable area of the phase diagram in a stable mixture (Abhyankar et al., 2011).

As mentioned above, different KGM concentrations and pH values are factors that determine the stability of emulsions. In the present study, the microscopic image of emulsion prepared at pH 7, 8, 9, and 10 and 0.04% KGM concentration were chosen, as shown in Figure 1. All emulsions were stable with homogeneous CLSM image after mixing (day 0). During storage, phase separation of emulsion could be observed. Emulsion stability can be determined by shape, size, and connection of the aggregate structure by phase separation (Abraham, 2012). At day 10, the microstructure of 0.04% KGM-stabilized emulsion was stable due to the influence of pH. It was found that lowering the pH resulted in an increase in the size of the aggregate and the shape of aggregate changed from isolated shape to interconnected shape, which was observed in the emulsion at pH 7 compared to the emulsion at pH 10.

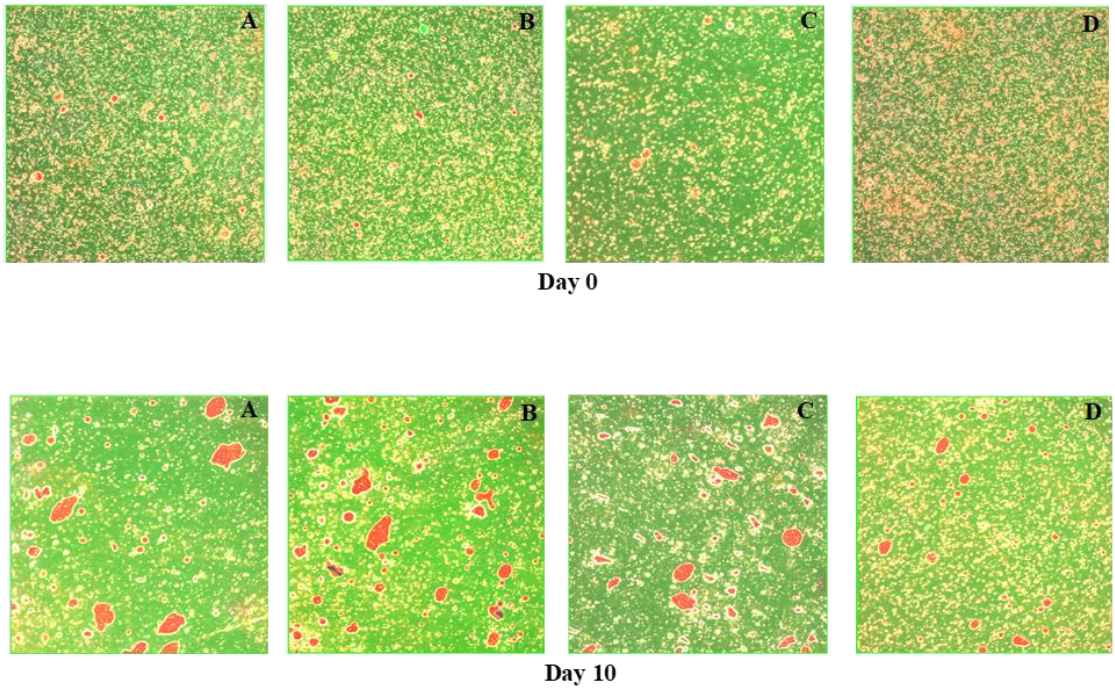

Figure 1 Microscopic image of emulsion at 0.04% KGM concentration as observed by confocal laser scanning microscopy (CLSM). A, B, C, and D represent the pH emulsion at pH 7, 8, 9, and 10, respectively.

5. Conclusion

The ability to stabilize fish oil emulsion in milk by KGM is dependent on the pH and concentration of the KGM solution. The emulsion stability in this study was improved by reducing the concentration of the KGM solution and increasing the pH. The highest stability of the emulsion was at 0.02% and 0.04% KGM and at pH 9 and 10. The mechanism by which KGM stabilizes the emulsion obtained with the use of different conditions is likely due to a combination of steric and electrostatic stabilization. This study reveals that KGM concentrations and pH influence protein-polysaccharide interactions. Improved stability of O/W emulsion model was obtained by structuring the water phase with KGM, which is a health-beneficial polysaccharide. The findings from this study provide insights from creating food products containing emulsions by modifying the water phase structure of the emulsion with naturally edible biopolymer. Moreover, this study provides information that may facilitate the design of emulsion-based delivery systems for polyunsaturated lipids. Further studies on the effects of other components in milk, such as calcium, will provide further information on the stability of the emulsion.

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7. References

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